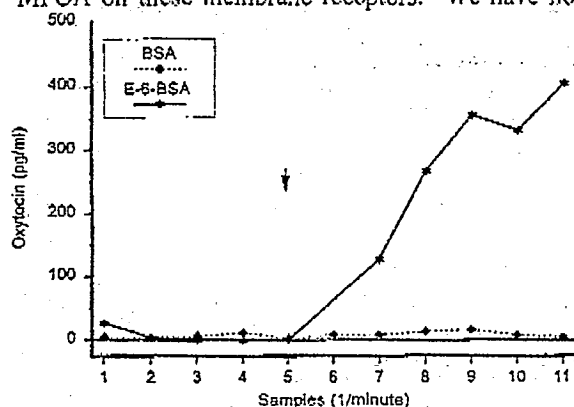


Steroid Effects at the Membrane Level on Oxytocin Release and Receptors

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The ovarian steroid estradiol (E) has effects on the oxytocin (OT) system at several levels. However, it has become apparent in the course of immunocytochemical and *in situ* hybridization work that E affects OT immunoreactive levels in brain areas and under conditions that are not explained by genomic actions of the steroid. Therefore, we began entertaining the postulate that at least some of the effects of E on OT systems were mediated at the level of the plasma membrane. An important area for OT-induced enhancement of female sexual receptivity is the medial preoptic area (MPOA): infusions of OT there enhance¹ and infusions of antagonists block receptivity². We have discovered there is a high density of steroid membrane receptors that are heterogeneous, i.e. have low- and high-affinity sites³. We also found that the G-protein antagonist GTPγS inhibited ligand binding while cholesta toxin (CTX) altered binding suggesting coupling of these receptors to G proteins. This presents the opportunity for steroids to act in the MPOA on these membrane receptors. We have now found that steroids act on two aspects of the OT



system in the MPOA by membrane-associated mechanisms: 1) E induces rapid release of OT from homogenates from the MPOA-hypothalamus and 2) steroids convert OT receptors (OTR) from a low- to a high-affinity state in plasma membrane preparations. Estradiol conjugated to bovine serum albumin at position 6 (E-6-BSA) released oxytocin (OT) from homogenates of the MPOA-medial hypothalamus within minutes of its superfusion (see Figure). Using a superfusion system in which synaptosome-containing homogenates were layered onto acridisols maintained at 37°C, we have found that E-6-BSA (100 ng/μl) superfusions significantly elevated OT release within minutes. In contrast, superfusion of the same concentration of BSA (see Figure) or progesterone-3-BSA (P-3-BSA) had no effect on

OT release. While superfusing homogenates with augmented levels of K⁺ had no effect on OT release itself, superfusing E-6-BSA with these concentrations of K⁺ consistently increased OT release. This is the first demonstration that E-6-BSA increases OT release in a nucleus-free medium. We have also used a radioligand antagonist for OT (¹²⁵I-OTA) that is specific for OTR to identify both low- (K_i = 19.1 ± 7.2 nM) and high-affinity OTR (K_i = 0.2 ± 0.06 nM). Using a range of OT (0.2 to 200 nM) to compete off a single concentration of ¹²⁵I-OTA we found that MPOA-AH and MBH membrane fractions demonstrated heterogeneity, i.e. there are two affinity states in membranes from OVXed rats. Interestingly, *in vitro* treatment with E-6-BSA converted the low-affinity MPOA ¹²⁵I-OTA binding sites to high affinity, suggesting that part of the effect of steroids on OTR is mediated at the membrane level. In a recent review⁴, we present evidence that at 37°C CTX also affected ¹²⁵I-OTA binding in a way similar to the effect of CTX on ¹²⁵I-P-3-BSA binding; i.e. CTX converted binding from a two- to a one-site model. This suggests that not only are G proteins involved with OT binding to OTR but that both ¹²⁵I-P-3-BSA and ¹²⁵I-OTA binding sites are CTX-sensitive. We now have a non-genomic mechanism whereby steroids affect OT systems in the MPOA to control reproductive behaviors.

References: 1. Caldwell, J. D., Jirikowski, G. F., Greer, E. R. and Pedersen, C. A. *Behavioral Neuroscience* 103: 655-662, 1989; 2. Caldwell, J. D., Johns, J. M., Fagg, B. M. and Pedersen, C. A. *Hormones & Behavior* 28: 288-302, 1994; 3. Caldwell, J. D., Walker, C. H., Fagg, B. M., Pedersen, C. A. and Mason, G. A. *Brain Research* 693: 225-232, 1995; 4. Caldwell, J. D., Walker, C. H., O'Rourke, S. T., Fagg, B. M., Morris, M. and Mason, G. A. *Hormone and Met. Res.* (in press) 1996.